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HIV-1 superinfection can occur in the presence of broadly neutralizing antibodies

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ABSTRACT

Background: Superinfection of individuals already infected with HIV-1 suggests that pre-existing immune responses may not adequately protect against re-infection. We assessed high-risk female sex workers initially infected with HIV-1 clades A, D or A/D recombinants, to determine if HIV-1 broadly neutralizing antibodies were lacking prior to superinfection.

Methods: Six superinfected female sex workers previously stratified by HIV-1 high-risk behavior, infecting virus clade and volunteer CD4 counts were evaluated at baseline (n = 5) and at 350 days post-superinfection (n = 6); one superinfected volunteer lacked pre-superinfection plasma. Retrospective plasmas were assessed for neutralization of a multi-clade panel of 12 HIV-1 viruses before superinfection, and then at quarterly intervals thereafter. Similarly stratified singly infected female sex workers were correspondingly assessed at baseline (n = 19) and 350 days after superinfection (n = 24). Neutralization of at least 50% of the 12 viruses (broad neutralization), and geometric means of the neutralization titers (IC_{50}) were compared before and after superinfection; and were correlated with the volunteer HIV-1 superinfection status, CD4 counts, and pseudovirus clade.

Results: Preexisting broad neutralization occurred in 80% (4/5) of the superinfected subjects with no further broadening by 350 days after superinfection. In one of the five subjects, HIV-1 superinfection occurred when broad neutralization was lacking; with subsequent broadening of neutralizing antibodies occuring within 9 months and plateauing by 30 months after detection of superinfection. Clade B and C pseudoviruses were more sensitive to neutralization (13; [87%]); and (12; [80%]) than the locally circulating clades A (10; [67%]) and D (6; [40%]), respectively (p = 0.025). Low antibody titers correlated with clade D viruses and with >500 CD4 T cell counts, but not with the superinfection status.

Conclusion: These data demonstrate that HIV-1 superinfection can occur both in the presence, and in the absence of broadly neutralizing antibodies.

1. Introduction

HIV-1 infected individuals who regularly practice unprotected sex with multiple sexual partners are prone to superinfection with new HIV-1 strains. This has been more frequently reported in early HIV-1 infection when host responses are relatively lower [1,2], implying that the natural anti-HIV immune responses may fail to prevent superinfection. This concept is supported by studies that associated superinfected individuals with lower broadly neutralizing antibody levels than their singly infected counterparts [2]. However, superinfection has also been shown in chronic HIV-1 disease where host immune responses are better established [3,4]. This suggests that pre-existing antibodies may not always adequately prevent further infection.

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Eliciting protective anti-HIV-1 antibodies is hindered by the continuous mutation of HIV-1 generating extensive diversity and eventual escape from the existing antibody response. Many monoclonal antibodies with significant coverage of the broad HIV-1 diversity have been isolated [5–8]. These prevented mucosal in macaques and humanized mice [9–12], and led to varying control of viraemia in human [13–15] and animal models [16–18], especially when used in combination [17]. Likewise, the subsequent 50% reduction in risk for HIV-1 infection of infected humans suggested potential protectiveness of pre-existing immunity [19]. HIV-1 superinfection is therefore as a good model for evaluating correlates of immune protection from HIV-1 infection. Here, we took advantage of existing stored plasmas from an incident female superinfected sex worker cohort [20] to evaluate relationships between neutralizing antibody responses and occurrence of HIV-1 superinfection.

2. Materials and methods

2.1. Study population

Quarterly plasmas from adult female sex worker participants of a high-risk HIV-1 behavioral cohort were collected between May 2008 and Dec-2013 [21,22]. Next-generation sequencing of partial p24 *gag* and *gp*41 envelope HIV genomic regions was used to screen the plasmas for HIV-1 superinfection, as described elsewhere [20,23]. Participants were categorized by the initial infecting HIV clade, HIV-1 risk behavior, and last CD4 T Cell count prior to superinfection. Trends of participants CD4 T cell counts over time were computed. Participant Superinfection timing was estimated as the median time between the last plasma with a single infecting strain and the first plasma with a newly acquired HIV-1 strain.

Of 85 screened drug-naïve female sex workers, seven with confirmed superinfection were evaluated for availability of stored specimens and screened for HIV-1 neutralizing antibodies at baseline (n = 5) and 350 days after study entry (n = 6). Singly infected participants were similarly assessed at baseline (n = 19) and at day 350 (n = 24). Ethical approval to conduct these studies was obtained from Uganda Virus Research Institute (UVRI) Research and Ethics Committee (REC), and from the Uganda National Council for Science and Technology (UNCST).

2.2. Preparation of pseudoviruses

Molecularly cloned pseudotyped viruses were generated in 293 T cells by co-transfection of specified envelope (*Env*) plasmids with *Env*-defective backbone plasmids (pSG3ΔEnv), and titrated in TZM-bl cells, as described elsewhere [24,25]. Plasmids were obtained from Prof Lynn Morris' lab, and from the National Institutes of Health AIDS Research and Reference Reagent Program. Neutralisation of a twelve-virus panel of tier II viruses comprising clade A, (Q23.17; Q769.d22 and Q842.d12); clade B (TRO.11, 6535.3 and RHPA); clade C (ConC; CAP45.G3 and Du156), and D viruses (QA013.H1; QB857.B3; and QD435.B5) was assessed. Where specimen volumes allowed, the effect of substitutions N160K and N33A at the envelope glycan sites known to be critical for broadly neutralization was evaluated to map specificities of the neutralizing antibody response.

2.3. Neutralization assays

Reduction in virus infection of target cells was quantified using Tat-regulated firefly luciferase reporter gene expression, as described elsewhere [25,26]. Briefly, duplicate wells of heat-inactivated, threefold serially diluted plasmas ranging from 1:40 to 1:1080 were screened for neutralization of a 12-virus panel. Wells with cells and media only were used for quantifying background luminescence. Virus controls were wells containing virus-infected cells, but lacking test plasmas. Wells with a strain of Vesicular Stomatitis Virus G (SVA.MLV) that is unrelated to HIV were included to screen out non-specific reactivity, and potential exposure to anti-retroviral drugs. Positive controls were plasmas predetermined to neutralize specified viruses.

Neutralization of HIV-1 was determined from linear interpolation of plotted virus infectivity curves as 50% inhibitory titres (IC_{50}). Inhibitory titres were computed as reciprocals of plasma dilutions that reduced relative luminescence in test wells by 50% compared to that in virus control wells. The assay lower detection limit was 40; therefore, titres below 40 were considered undetectable and were arbitrarily assigned to be 20 for ease of analysis. Inhibitory titres of 40 and above were confirmed in a repeat test, and an average titre computed. Plasma with titres of 1080 and above were retested in threefold serial dilution ranging from 1:20 to 1:43,740, to define the endpoint titre. All neutralization titres used are derived from means of two independent tests.

Neutralization breadth was determined as the proportion (%) of the 12 viruses inhibited. Stricter criterion was used to determine titres for neutralizing breadths, inhibitory titres below 50 were considered unreactive. Plasmas that neutralized at least 50% of the viruses were considered to be broadly neutralizing. Geometric means of the neutralization breadths and neutralization titres were correlated with SI status, CD4 counts, and infecting virus clade.

2.4. Statistical analyses

Statistical analyses and graphical presentations were performed using Graph Pad 5.0 (GraphPad Software, Inc., San Diego, California USA). Neutralization breadths and geometric means of the titers (IC_{50} values) were compared at two time points: (i) pre-superinfection, as the closest time point to the visit at which superinfection was detected, and (ii) 350 days post-superinfection, as the earliest analysis time point after superinfection. Median matched values were compared using the Wilcoxon rank sum test. Geometric means of titres were compared using t-tests. Regression analyses and confidence intervals of neutralizing antibody means (across 12 viruses) were computed over time.

Regression analysis was also used to estimate the overall CD4 + T Cell slopes over time. Slope estimates were expressed as coefficients. Negative coefficients indicated decreasing trend of neutralizing antibodies/CD4 T Cell counts over time, and positive slopes indicate increasing trend over time. P-values of ≤ 0.05 indicated that the slope was significantly different from zero. Factors linked to SI status relative to one's CD4 count strata and HIV-1 risk behavior profiles (number of sexual partners) were assessed using a conditional logistic regression model. A normal interval regression model of generalized estimating equations was used to evaluate effects of SI status on absolute log10 inhibitory titres, after adjusting for CD4 counts, pre-SI breadth, neutralization titers and infecting clade.

3. Results

3.1. Study participants

We present profiles of HIV neutralizing antibody responses for six superinfected, and 24 singly infected drug naïve female sex workers. Median participation time since study entry for all six superinfected cases was 313 days (Interquartile range [IQR] 295–367). One superinfected volunteer lacked plasma prior to superinfection; thus, their pre-superinfection data constitutes five